We have cloned a new copper sensitive "\(\sigma^{\text{III}}\)"-regulatory system: pMxMoR, which is repressed by copper (J. Scanlon et al., 2009).

Two new fluorescent proteins, EBFP2 and iRFP, have also been cloned.

We have also begun work on generating a data sheet with the following categories of information represented:

- General Information (Author, date, Brief abstract, links to relevant sources)
- Part Information (Device design and composition, Assembly method(s))
- Growth/Measurement Conditions (Specific experimental conditions, List of assays used)
- Data Analysis (Gel images, Sequencing data, Flow cytometry analysis, Other assay analysis)

We plan to pull this information from our Clotho database so any Clotho user can generate these data sheets for their parts.

### CURRENT AND FUTURE WORK

- We are building more Level 0 parts and will submit them to the Registry when confirmed
- We will make fusion proteins to combine functional genes and fluorescent reporters using MoClo
- We are modifying the growth parameters for our characterization experiments to include 96-well plates
- We will test our parameters on multiple flow cytometers
- We are writing the RFC standard for MoClo and will submit that to the Biobricks Foundation within the next month
- We will complete and share the code to make our data sheet so future iGEM teams that use Clotho can easily pull together all of the information required for their Registry pages

### OUR CONTRIBUTIONS TO iGEM

- Converted 29 BioBrick parts into 31 MoClo Parts (Foundation for converting iGEM to MoClo)
- Contributed 4 new parts, for a total of 35 parts
- Characterization workflow for circuits with fluorescent proteins in development with shown success

### SHARING: MoClo Kit

- 18 Promoters
- 5 RBS
- 11 Genes
- 1 Terminator
- 35 level 0 MoClo Parts
- 990 different Level 1 MoClo combinations possible

This figure shows the number of DNA parts in our MoClo Kit and the power of MoClo as an assembly technique.

### BUILDING: Converting BioBricks

**We converted 31 BioBricks and 4 new parts into Level 0 MoClo parts to create a library of MoClo parts.**

For BioBrick parts >100bp, we used a standard PCR methodology.

**Level 0 Primer Design for Standard PCR**

<table>
<thead>
<tr>
<th>Forward: AGGAAGATTAC(TGAGAC)GGG</th>
<th>Reverse: [last 24bps of part]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward: AGGAAGATCA</td>
<td>Reverse: [first 24bps of part]</td>
</tr>
</tbody>
</table>

We used a ligation PCR methodology for sequences <100bp in length (J. Lee et al., Biotechniques. 2004).

All of our parts and primers are stored in a Clotho database (Kia et al., Methods in Enzymology 2011).

### BUILDING: Modular Cloning

*Modular Cloning, or MoClo, is a relatively new assembly method based on Golden Gate introduced in 2011 by Ernst Weber et al., which uses Type IIS restriction enzymes (we used BpiI and BsaI) to generate 4bp overhangs. These 4bp overhangs are called fusion sites, which must match between parts in order to ligate them together.*

**This allows the user to ligate up to six DNA parts together in a one-pot reaction, cutting down the time it takes to build large circuits dramatically (shown at right).**

There are three Levels of MoClo Parts (shown below):

- **Level 0**: Basic (ex: promoter, RBS, CDS, etc.)
- **Level 1**: Transcriptional unit (up to 6 Level 0 Parts)
- **Level 2**: Composite of up to 6 Level 1 parts

### SHARING: Data Sheet

All parts listed above were confirmed with sequencing.

- We have also submitted all of our Level 0 MoClo parts and cloning vectors for Level 0, Level 1 and Level 2 to the Registry as part of our MoClo Kit.
- It is our hope that future iGEM teams will contribute to this library of Level 0 MoClo parts so iGEM can move away from binary assembly and towards this modular, one-pot assembly technique.

### CHARACTORIZING: Suggested Workflow

We have begun developing a flow cytometry workflow for the characterization of genetic circuits that contain fluorescent proteins in *E. coli* (shown below).

- Parameters that often vary between labs is the time of growth, culture shaking and lasers, filters, and settings used in the flow cytometer for measurement.
- We are in the process of testing these variables to determine the parameters that will work best for the genetic circuits we have built using BioBricks.